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(54) Title: MONOCLONAL ANTIBODIES WHICH BIND TO SECRETED AND MEMBRANE-BOUND IgE, BUT NOT TO IgE ON BASOPHILS (57) Abstract Disclosed is the murine monoclonal antibody ("mAb") TES-C21, and the chimeric mouse/human mAb TESC-2, which has its variable regions derived from TES-C21, and has human ($\gamma 1, \kappa$) constant regions. Both TES-C21 and TESC-2 bind specifically to IgE and are not reactive with other heavy chain isotypes. Both mAbs bind specifically to IgE secreting B cells, and are not reactive with other leukocytic cell types. Neither antibody binds to IgE bound to Fc ϵ R11 and both mAbs inhibit the binding of IgE to the low affinity Fc ϵ R11 receptor on B cells. Neither mAb includes histamine release from human basophils. TESC-2 inhibits the binding of IgE to basophils. These properties make these mAbs well-suited for use in human allergy therapy.		

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5 **MONOCLONAL ANTIBODIES WHICH BIND TO SECRETED AND
MEMBRANE-BOUND IgE, BUT NOT TO IgE ON BASOPHILS**

Background of the Invention

The immediate-type hypersensitivities, such as extrinsic asthma, hay fever, and allergic responses to certain foods or drugs, are mediated primarily by one isotype of the immunoglobulins, *i.e.*, IgE. In an IgE-mediated allergic response, the allergen binds to the IgE which is bound to receptors on the surfaces of mast cells and basophilic leukocytes (basophils). The binding of the allergen causes cross-linking of the surface IgE molecules and hence the underlying receptors (FcεR) for the Fc portion of IgE, thereby triggering the release of pharmacologic mediators such as histamine, the slow-reacting substance of anaphylaxis (SRA), and serotonin. The release of these mast cell and basophil products causes the pathological reactions and symptoms of allergy.

IgE is secreted by a particular class of B cells, which also express IgE on their surface. In individuals sensitized to specific allergens, the allergen-specific IgE is continuously produced by these B cells. Nevertheless, individuals who have no secreted IgE in their systems (and no IgE producing B cells) appear to live normally, indicating that IgE is not essential in the immune response. It seems, therefore, that eliminating or suppressing secreted IgE by either binding it directly with an antibody or by depleting

IgE producing B cells, would be a viable therapy for allergy. Monoclonal antibodies (and derivative and related products) which bind specifically to secreted IgE and to the IgE producing B cells could be used in such a suppression or elimination process. The immune system's regulatory, cytolytic or cytotoxic mechanisms can be used to suppress or destroy the B cells which are bound by monoclonal antibodies. Other immune mechanisms can eliminate the secreted IgE which is bound by the monoclonal antibodies.

IgE binds to the Fc ϵ R receptors on the surface of basophils and mast cells very strongly, with an association constant, K_a , of about 1×10^{10} liter/mole. Even though IgE is not synthesized by basophils and mast cells, the very strong and stable association of IgE with Fc ϵ R means that IgE is virtually always present and exposed on the surface of these cells. Thus, an immunotherapeutic agent which targets IgE must not react with the IgE on basophils and mast cells, in order to avoid cross-linking this IgE and the underlying Fc ϵ R and thereby triggering an allergic reaction.

Summary of the Invention

The monoclonal antibody ("mAb") TES-C21 is a murine monoclonal antibody, and TESC-2 is a chimeric mouse/human mAb with its variable regions derived from TES-C21, and with human ($\gamma 1$, κ) constant regions. Both TES-C21 and TESC-2 bind specifically to IgE and are not reactive with other heavy chain isotypes. Both mAbs bind to IgE secreting B cells, and

neither induces histamine release from human basophils. Both mAbs also inhibit the binding of IgE to the low affinity FcεRII receptor, which is present on B cells producing all of the various heavy chain isotypes.

These properties make these mAbs well suited for use in allergy therapy. They will bind specifically to secreted IgE, and the secreted IgE can then be eliminated by the immune system. They also bind specifically to IgE expressed on the surface of IgE-producing B cells, and these B cells can then be suppressed or destroyed by the immune system's regulatory, cytolytic or cytotoxic mechanisms without affecting B cells producing other immunoglobulin isotypes. Neither of these mAbs induces histamine release, so they will not cause any adverse affects upon administration. Also, because neither of these mAbs binds to IgE on the low affinity FcεRII receptors on B cells, few of the B cells producing immunoglobulin isotypes other than IgE will be adversely affected by administration of these mAbs.

The epitope which is bound by the TES-C21 and TESC-2 mAbs is believed to be located on the Fc region of the IgE molecule, near to the binding site of FcεR. The invention will now be described in further detail with reference to the drawings.

Brief Description of the Drawings

Fig. 1 shows a comparison of the binding of TES-C21 and TESC-2 to IgE which is bound to microtiter plates.

Fig. 2 shows a comparison of the relative affinity of the binding of

TES-C21 and TESC-2 to IgE which is bound to microtiter plates.

Fig. 3 shows a comparison of the binding of TES-C21 and TESC-2 to IgE-secreting cells.

Fig. 4 shows a comparison of the inhibition of the binding of IgE to
5 FcεRII by TES-C21 and TESC-2.

Detailed Description of the Preferred Embodiments and Their Manner and Process of Making and Using

The epitopes targeted by the TES-C21 and TESC-2 mAbs are unique in that they are present on IgE bearing B lymphocytes but not on mast cells
10 or basophils. The epitopes are believed to be located at or near the binding site for FcεR, and to be obscured when IgE is bound to FcεR on basophils and mast cells. These mAbs are specific for the IgE-secreting B cells, and did not bind to B cell lines secreting other Ig isotypes, to a T cell line or a monocyte-like cell line, or to peripheral blood mononuclear cells.

15 The TES-C21 and TESC-2 mAbs also do not bind to IgE on the low affinity FcεRII receptors on B cells, thereby reducing the destruction or regulation of B cells which produce isotypes other than IgE.

The TES-C21 and TESC-2 mAbs can be used in any way in which the mAbs described in published International Application PCT/US88/04706
20 can be used. These uses include diagnostic uses such as identifying and enumerating IgE-bearing B cells in mixed leukocyte populations, and quantifying levels of IgE in serum samples. These uses also include human allergy therapy, for which the chimeric TESC-2 mAb is preferred. The

mAbs can be systemically administered, preferably intravenously or intramuscularly, as free antibodies to patients afflicted with IgE-mediated allergy in amounts sufficient to down-regulate or eliminate IgE-producing cells, and/or amounts sufficient to bind to secreted IgE.

5 TESC-2 is of IgG1 subclass, and therefore mediates antibody dependent cellular cytotoxicity ("ADCC") of B cells expressing IgE on their surface. These IgE-expressing B cells also secrete IgE. Therefore, TESC-2 can be used to reduce or eliminate the IgE producing B cells. Nevertheless, TES-C21 could also be used to down-regulate B cells producing IgE through
10 other regulatory immune mechanisms.

While TES-C21 and TESC-2 can be used for *in vivo* therapeutic applications, they may also be used in extra-corporeal *ex-vivo* applications. The IgE-bearing B cells in the circulation of the patients can be removed by an affinity matrix (antibody immobilized on a solid phase) which is
15 conjugated with these mAbs.

TESC-2 is also preferred for *in vivo* use because it is a humanized antibody, and it therefore less immunogenic than the wholly murine mAb TES-C21. Such humanized mAbs are less likely to evoke an immune or allergic response. It is noted that such a response could deplete the
20 antibodies which are administered before such antibodies could function to bind to the secreted or membrane-bound forms of IgE, thereby reducing the effectiveness of the therapy.

The mAbs TES-C21 and TESC-2 can also be used as carrier agents of cytotoxic drugs or for delivering an effector substance, by conjugating the mAbs to these substances. A toxin-antibody conjugate will bind and directly kill B cells producing IgE, but not B cells producing other isotypes which do not express IgE on their surfaces. These toxins are cytolytic or cytotoxic agents, including cytotoxic steroids, gelonin, abrin, ricin, *Pseudomonas* toxin, diphtheria toxin, pokeweed antiviral peptide, tricarhecum, radioactive nuclides, and membrane-lytic enzymes (such as phospholipase). The method of making these conjugates is described in International Application PCT/US88/04706.

The TES-C21 and TESC-2 mAbs can also be used in combination with factors which enhance ADCC, such as granulocyte monocyte-colony stimulating factor ("GM-CSF") and monocyte-colony stimulating factor ("M-CSF"). These combination therapies are also described in International Application PCT/US88/04706.

Immunotherapies employing the mAbs of the invention can be used in combination with conventional desensitization immunotherapy. For example, desensitization with allergen can be performed in conjunction with the administration of either the mAbs of the invention or antibody-toxin conjugates discussed above, to down-regulate or substantially eliminate IgE producing cells.

Desensitization induces IgG production against the

allergen/immunogen. Inducing such IgG production may be most effective as an allergy therapy when IgE-producing B cells are substantially depleted. The combination of antibody and desensitization therapy is attractive because although the IgE-producing B cells may only be temporarily
5 depleted (for a few weeks or months) by the mAbs of the invention, and will eventually re-populate, the desensitization effect may last much longer.

The TES-C21 and TESC-2 mAbs can also be used in making anti-idiotypic antibodies, as described in International Application PCT/US88/04706. These anti-idiotypic antibodies can be used to actively
10 immunize against IgE and induce endogenous formation of antibodies against the epitope on IgE which TES-C21 and TESC-2 bind to. The induced antibodies will deplete IgE-producing B cells and bind to secreted IgE, as described in International Application PCT/US88/04706.

The TES-C21 and TESC-2 mAbs can also be used in making
15 derivative antibodies which draw cytotoxic cells such as macrophages or cytotoxic T cells toward the targeted IgE-expressing B cells. These derivative antibodies, which are useful in allergy therapy, include bi-specific antibodies having a specificity for a receptor of a cytotoxic cell and a specificity for the IgE expressing cells. Such hybrid bi-specific antibodies can
20 include two different Fab moieties, one Fab moiety having antigen specificity for the targeted B cells, and the other Fab moiety having antigen specificity for a surface antigen of a cytotoxic cell, such as CD3 or CD8. The bi-

specific antibodies of the invention can be a single antibody having two specificities, or a heteroaggregate of two or more antibodies or antibody fragments. See, e.g., C. Reading, U.S. Patent Nos. 4,474,893 and 4,714,681; Segal *et al.*, U.S. Patent No. 4,676,980.

5 Making the mAbs of the Invention

 The mAbs TES-C21 and TESC-2 are made by techniques well-known in the art, which are described in International Application No. PCT/US88/04706. Briefly, male Balb/c mice were immunized several times with polyclonal human IgE from sera (provided by Ventrex), where the IgE
10 was combined with a suitable adjuvant. Mice were sacrificed after the last injection of immunogen and the spleens were removed for preparing single cell suspensions for fusion with myeloma cells. The spleen cells were fused with Sp2/0 cells using a fusion mixture of polyethylene glycol 1450 (Kodak), CMF-PBS and DMSO. DMEM was added after the cell suspensions were
15 combined.

 The hybridomas resulting from the fusion were then screened by enzyme-linked immunosorbent assay (ELISA) against human IgE bound to an Immulon 2 plate. One of these hybridomas produced TES-C21.

 Using standard methods for making chimeric antibodies, Sp 2/0 cells
20 were co-transfected with the variable regions of TES-C21 H and L-chains, and human γ 1 and κ constant regions, and aliquoted into 96 well plates for selection. Supernatants were screened for secretion of human IgG which

bound to human IgE.

The transfectoma cells were adapted to growth in serum-free medium. TESC-2 was then purified from medium of confluent cultures using an immobilized protein A column. The hybridoma cell line producing the mAb
5 TESC-2 was deposited pursuant to the Budapest treaty at the American Type Culture Collection, Rockville, Maryland, on March 26, 1991 under accession number _____.

Properties of the mAbs of the Invention

The mAb TES-C21 was found, on testing by ELISA, to be specific for
10 human IgE, and not to react with IgG, IgM, IgA, IgD, human serum albumin, transferrin or insulin. TES-C21 bound equally well to various human IgE molecules. TES-C21 bound to the IgE-secreting cell lines SKO-007, U266 and SE44 in a dose-dependent manner. But TES-C21 did not bind to human B cell lines bearing surface IgM, IgD, IgG, or IgA, or to a T
15 cell line or to the parent murine cell line of SE44, or to a murine cell line secreting chimeric human IgG. TES-C21 does not bind to IgE on low affinity FcεRII receptors, and does not induce histamine release from freshly prepared human blood basophils, on which the FcεR are armed with IgE. At 10 μg/ml TES-C21 is able to inhibit completely the binding of IgE to
20 FcεRII.

As shown in Fig. 1, TESC-2 and TES-C21 bind equally well to IgE bound to microtiter plates by binding to antigen. Immulon 2 plates were

coated with gp120 peptide-ovalbumin conjugate and IgE-SE44 was bound to the immobilized antigen. TES-C21 or TESC-2 at the concentrations in Fig. 1 were added. Binding was detected using either horseradish peroxidase ("HRP")-conjugated goat antimouse IgG (for TES-C21) or HRP-goat antihuman IgG, Fc (for TESC-2). The values shown in Fig. 1 represent the means of duplicates from one representative of three experiments.

As shown in Fig. 2, TESC-2 and TES-C21 have the same relative affinity for IgE bound to microtiter plates by binding to antigen. Immulon 2 plates were coated with gp120 peptide-ovalbumin conjugate and IgE-SE44 was bound to the immobilized antigen. TES-C21 and TESC-2 at the concentrations indicated in Fig. 2 were added and preincubated for 1 hour before adding 0.22 μ g/ml of biotinylated TES-C21. Binding of biotinylated TES-C21 was detected using horseradish peroxidase-conjugated streptavidin. Values shown in Fig. 2 represent the means of duplicates from one representative of three experiments.

TESC-2 and TES-C21 bind equally to IgE-secreting cells, as shown in Fig. 3. Cells were incubated at 2×10^6 cells/100 μ l PBS-1% goat serum in the indicated antibody concentrations at 0° for 30 min. Binding of TES-C21 was detected using FITC-goat (Fab')₂ antimouse IgG; binding of TESC-2 was detected using FITC-goat (Fab')₂ antihuman IgG. Binding was quantitated by fluorescence flow cytometry using a Coulter Epics V. The FITC intensity gate was set to yield 10% \pm 0.5% positive cells in the

absence of primary immunoglobulins. The data shown in Fig. 3 represents the net increase in percent positive cells.

It was found that neither TES-C21 nor TESC-2 bound to IgE which was bound to low affinity IgE receptors. Some B cells (regardless of the isotypes of their membrane-bound immunoglobulins), T cells, monocytes, and platelets express a low-affinity IgE receptor, CD23, on their surface. The possibility that TESC-2 recognized IgE complexed with CD23 was studied using cells of an IgG-secreting human lymphoblastoid line, IM-9. The presence of CD23 on IM-9 cells was confirmed by their strong staining with anti-Leu 20, a MAb specific for CD23. IM-9 cells were incubated with 5 or 10 μ g/ml of human IgE, washed, and then incubated with biotin-labeled TESC-2 or a positive control anti-IgE mAb TES-19, followed by FITC-streptavidin and analyzed by flow cytometry.

As seen in Fig. 4, both chimeric TESC-2 and murine TES-C21 inhibit binding of IgE to Fc ϵ RII. The mAb were preincubated at the indicated concentrations with 20 μ g IgE-SE44 for 1 hour at 37° before addition of IM-9 cells bearing Fc ϵ RII. Binding of IgE was detected using biotinylated TES-19 and FITC-streptavidin and quantitated by fluorescence flow cytometry. HEM7 is a control mAb that does not bind to serum IgE and does not affect IgE binding to Fc ϵ RII.

It may be assumed that immune complexes of TESC-2 and IgE were formed during their preincubation in these experiments. These immune

complexes also did not bind to FcεRII, as confirmed using biotin-labeled TESC-2 or FITC goat anti-human IgE (with TES-C21).

As shown in Table I below, neither TESC-2 nor TES-C21 induces histamine release from freshly prepared human blood basophils, on which the FcεR are armed with IgE. Due to the variable release of mediators from basophils of different donors, the mAbs were examined at multiple concentrations on basophil preparations from multiple donors. No induction of histamine release by TESC-2 or TES-C21 was observed.

BLE I

		<u>Net Histamine Release</u>			
<u>Antibody</u>	<u>Concentration μg/ml</u>	<u>Donor 1</u>	<u>Donor 2</u>	<u>Donor 3</u>	<u>Donor 4</u>
Polyclonal goat anti IgE TESC-2	0.1	70	64	55	81
	0.4	0	2		
	2	0	3		
	10	0	2		
	50	0	2		
TES-C21	0.4			1	0
	2			1	0
	10			0	0
	50			1	0

To address the possibility that TES-C21 and TESC-2 might bind to basophils but be unable to crosslink the receptors to induce histamine release, a secondary antibody was used for crosslinking. Since antihuman IgG alone can induce histamine release, only the murine TES-C21 was used

in these experiments. The crosslinking goat antimouse IgG enhances histamine release induced by suboptimal concentrations of control anti-IgEs. TES-C21 did not induce histamine under these very permissive conditions.

TESC-2 was further tested to determine whether it could block the binding of IgE to FcεRI receptors, and whether immune complexes of IgE and TESC-2 would bind to these receptors. To determine whether TESC-2 inhibits the binding of human IgE to FcεRI, human peripheral blood basophils that had been depleted of IgE by treatment at low pH were reloaded or sensitized with SE44-derived chimeric IgE reactive to a peptide antigen. Functional binding of SE44 IgE was tested by histamine release induced by the polyvalent R15K peptide-ovalbumin conjugate to which the variable region of IgE-SE44 binds. Preincubation of IgE-SE44 with TESC-2 inhibited IgE binding to FcεRI (Table 2). Binding of SE44 IgE was also inhibited when basophils were incubated with another IgE (PS) before exposure to IgE-SE44. It may be assumed that immune complexes of TESC-2 and IgE were formed during the preincubation and did not cause the release of histamine. The experimental conditions and the results of these experiments are summarized below in Table 2.

Because the conditions for IgE loading on basophils and histamine release are different, additional experiments were done to confirm that immune complexes of TESC-2 and IgE did not induce histamine release.

TABLE 2

**Inhibition of IgE Binding to High-Affinity
IgE Receptors by TESC-2**

		<u>Net Histamine Release (% of total)</u>	
	<u>Conditions for Basophil loading with IgE-SE44</u>	<u>Challenge with R15K Peptide-Ovalbumin</u>	<u>Challenge with anti-IgE</u>
5	IgE-SE44 was not preincubated with TESC-2	37	66
	IgE-SE44 was preincubated with TESC-2	3	68
10	IgE-SE44 was preincubated with IgE-PS	0	63

The following is a summary of the key properties of the chimeric mAb TESC-2 and the murine mAb TES-C21, but it must be understood that the mAbs TES-C21 and TESC-2 both have other properties, some known and some unknown. Many derivative products and other uses for these mAbs are possible, and the invention, as defined by the claims below, includes all of these properties, products and uses, and also includes all equivalents of the subject matter of those claims.

KEY PROPERTIES OF TESC-2

- * Chimeric mAb with human ($\gamma 1, \kappa$) constant regions
- * pI: 5 bands, range 7.2 - 7.8, major band 7.45
- * Binds specifically to IgE with high affinity
- * Binds to IgE-secreting cells reaching more than 50% maximum binding at 1 μ g/ml

15

- * Does not bind to IgE bound to the low affinity IgE.Fc receptor
- * Inhibits binding of IgE to low affinity IgE.Fc receptor
- 5 * Does not induce histamine release from peripheral blood basophils
- * Inhibits the binding of IgE to the high affinity IgE.Fc receptor
- * Immune complexes do not bind to low affinity IgE.Fc receptor or induce histamine release from peripheral blood basophils
- 10

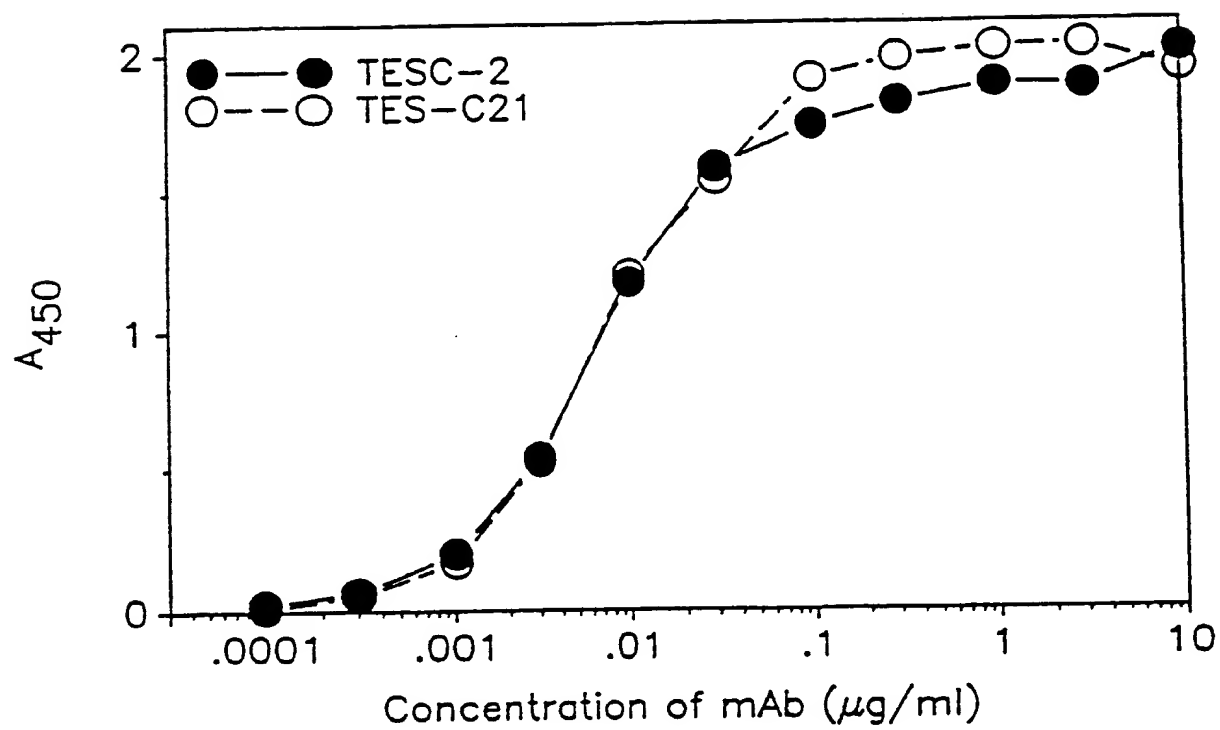
KEY PROPERTIES OF TES-C21

- * Murine mAb
- * Binds specifically to human IgE-secreting cells; not reactive with other cell types
- 15 * Does not bind to IgE on low affinity IgE.Fc receptor
- * Inhibits binding of IgE to low affinity IgE.Fc receptor
- * Does not induce histamine release under very permissive conditions
- 20

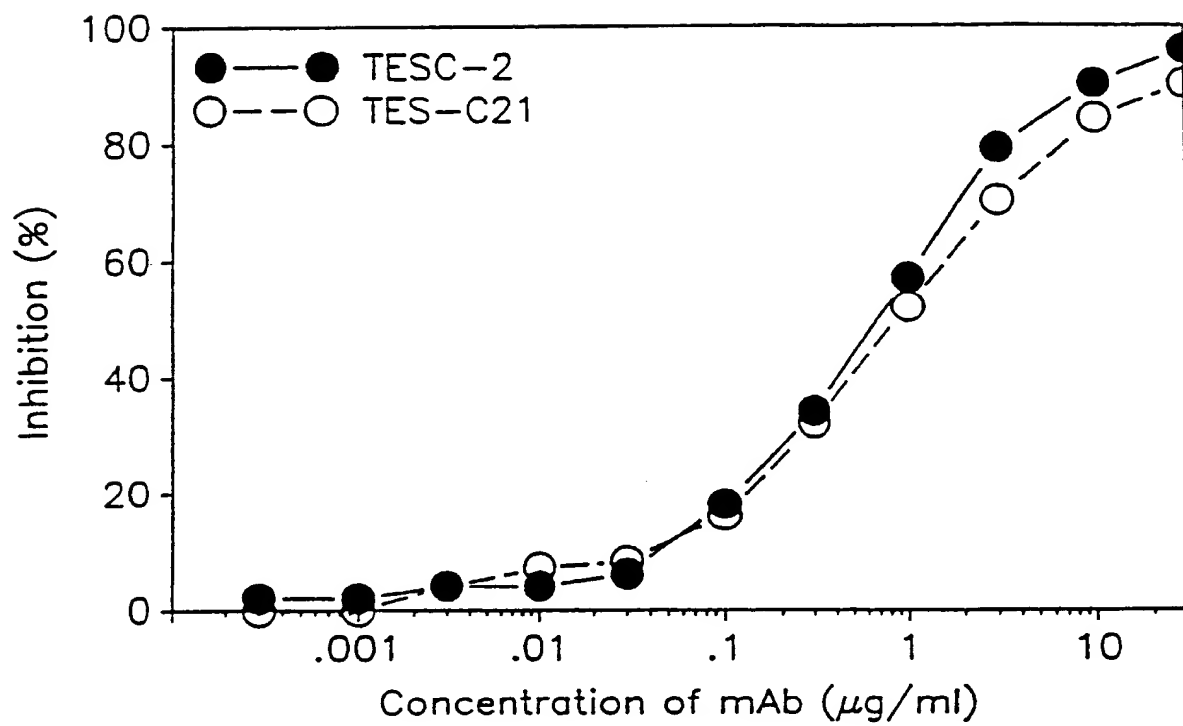
WHAT IS CLAIMED IS:

1. The monoclonal antibody TESC-2, or a monoclonal antibody with equivalent properties.
2. The cell line producing the monoclonal antibody TESC-2 or a
5 monoclonal antibody with equivalent properties.
3. An immunotoxin comprising the monoclonal antibody TES-C21 or TESC-2 conjugated with a cytotoxic or cytolytic agent.
4. The immunotoxin of claim 3 wherein the agent is selected from the group consisting of cytotoxic steroids, gelonin, abrin, ricin, Pseudomonas toxin,
10 diphtheria toxin, pokeweed antiviral peptide, tricathecums, radioactive nuclides, and membrane lytic enzymes.
5. Isolated DNA comprising functionally rearranged genes encoding at least a portion of the variable region of a light or heavy chain of the monoclonal antibody TES-C21.
- 15 6. A DNA construct comprising the DNA of Claim 5 linked to DNA encoding a human light or heavy chain constant region.

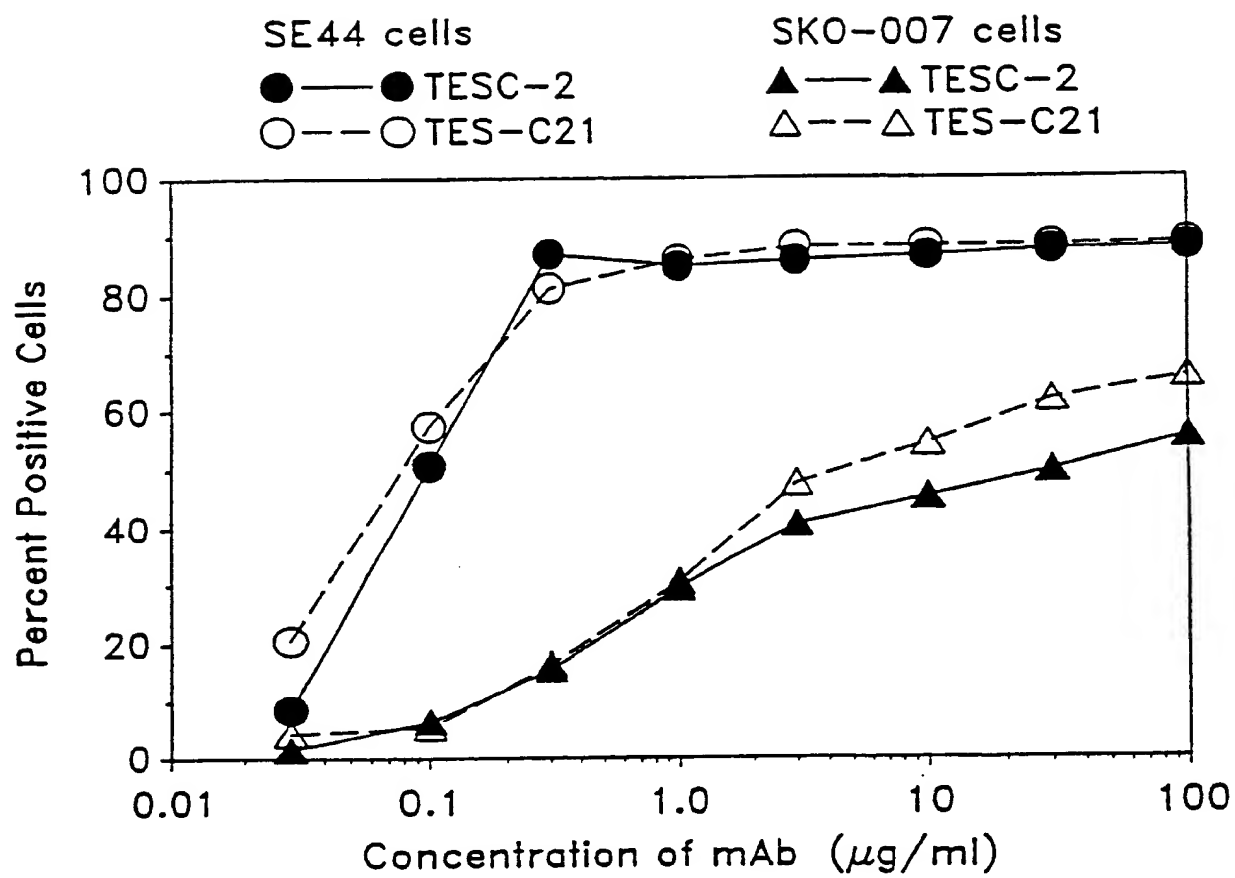
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**Fig. 1**

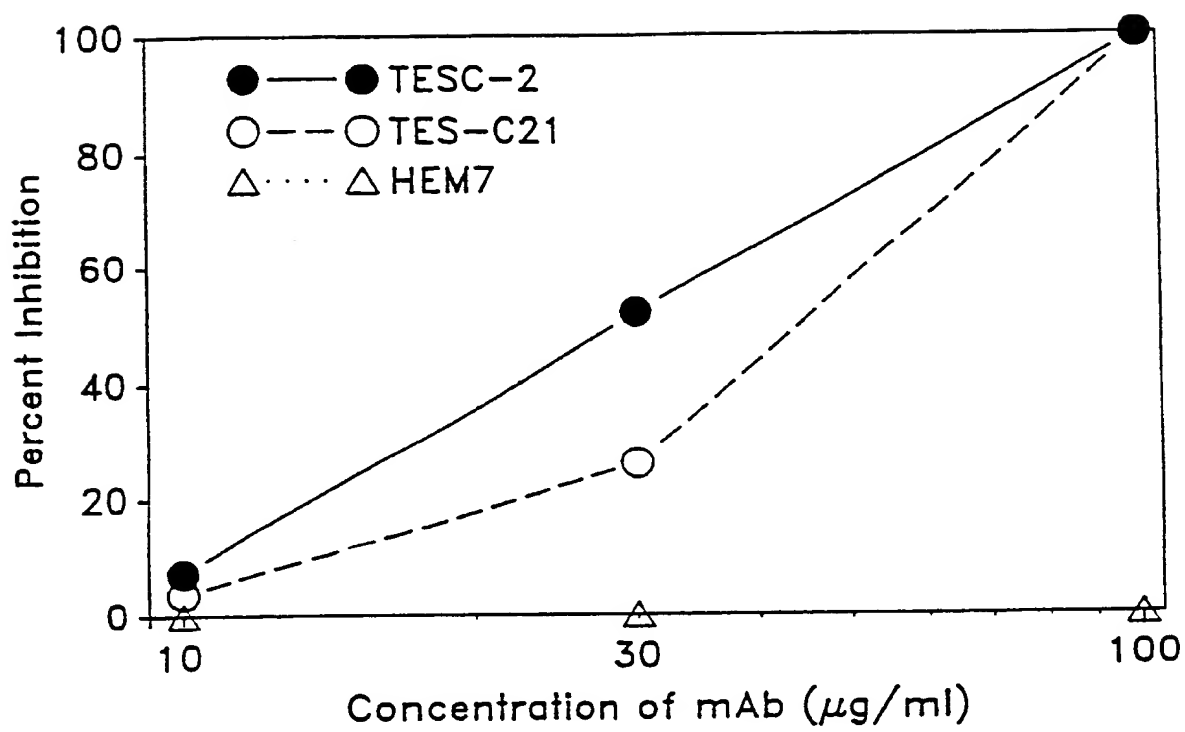
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**Fig. 2**

3/4




**Fig. 3**

4/4

**Fig. 4**

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US91/01991

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) ⁶ According to International Patent Classification (IPC) or to both National Classification and IPC IPC (5): A61K 39/40, 39/44 U.S. CL. 424/85.8																							
II. FIELDS SEARCHED <div style="text-align: center; border-top: 1px solid black; border-bottom: 1px solid black;">Minimum Documentation Searched ⁷</div> <table style="width: 100%; border-collapse: collapse;"> <tr> <th style="width: 25%; border: 1px solid black; text-align: left;">Classification System</th> <th style="border: 1px solid black; text-align: left;">Classification Symbols</th> </tr> <tr> <td style="border: 1px solid black; text-align: center; vertical-align: middle;">U.S.</td> <td style="border: 1px solid black;">424/85.8, 85.91, 86, 87</td> </tr> </table> <div style="text-align: center; border-top: 1px solid black; border-bottom: 1px solid black;">Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched ⁸</div>			Classification System	Classification Symbols	U.S.	424/85.8, 85.91, 86, 87																	
Classification System	Classification Symbols																						
U.S.	424/85.8, 85.91, 86, 87																						
III. DOCUMENTS CONSIDERED TO BE RELEVANT ⁹ <table style="width: 100%; border-collapse: collapse;"> <tr> <th style="width: 10%; border: 1px solid black;">Category [*]</th> <th style="width: 70%; border: 1px solid black;">Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²</th> <th style="width: 20%; border: 1px solid black;">Relevant to Claim No. ¹³</th> </tr> <tr> <td style="border: 1px solid black; text-align: center;">A</td> <td style="border: 1px solid black;">US, A, 4,714,759 (WHITAKER, JR.) 22 DECEMBER 1987 See entire document.</td> <td style="border: 1px solid black; text-align: center;">1-4</td> </tr> <tr> <td style="border: 1px solid black; text-align: center;">A</td> <td style="border: 1px solid black;">US, A, 4,845,042 (NEWMAN ET AL) 04 JULY 1989; See entire document.</td> <td style="border: 1px solid black; text-align: center;">1-4</td> </tr> <tr> <td style="border: 1px solid black; text-align: center;">A</td> <td style="border: 1px solid black;">US, A, 4,865,980 (STUART ET AL) 12 SEPTEMBER 1989 See entire document.</td> <td style="border: 1px solid black; text-align: center;">4, 5</td> </tr> <tr> <td style="border: 1px solid black; text-align: center;">A,E</td> <td style="border: 1px solid black;">US, A, 5,017,489 (PASTERNAK ET AL) 21 MAY 1991 See entire document.</td> <td style="border: 1px solid black; text-align: center;">1-4</td> </tr> <tr> <td style="border: 1px solid black; text-align: center;">A,E</td> <td style="border: 1px solid black;">US, A, 5,026,545 (SAINT-REMY ET AL) 25 JUNE 1991 See entire document.</td> <td style="border: 1px solid black; text-align: center;">1-4</td> </tr> <tr> <td style="border: 1px solid black; text-align: center;">A,E</td> <td style="border: 1px solid black;">US, A, 5,053,224 (KOPROWSKI ET AL) 01 OCTOBER 1991 See entire document.</td> <td style="border: 1px solid black; text-align: center;">1-4</td> </tr> </table>			Category [*]	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³	A	US, A, 4,714,759 (WHITAKER, JR.) 22 DECEMBER 1987 See entire document.	1-4	A	US, A, 4,845,042 (NEWMAN ET AL) 04 JULY 1989; See entire document.	1-4	A	US, A, 4,865,980 (STUART ET AL) 12 SEPTEMBER 1989 See entire document.	4, 5	A,E	US, A, 5,017,489 (PASTERNAK ET AL) 21 MAY 1991 See entire document.	1-4	A,E	US, A, 5,026,545 (SAINT-REMY ET AL) 25 JUNE 1991 See entire document.	1-4	A,E	US, A, 5,053,224 (KOPROWSKI ET AL) 01 OCTOBER 1991 See entire document.	1-4
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<div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <p>[*] Special categories of cited documents: ¹⁰</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 45%;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"Δ" document member of the same patent family</p> </div> </div>																							
IV. CERTIFICATION <table style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 50%; border: 1px solid black; vertical-align: top;"> Date of the Actual Completion of the International Search <div style="text-align: center;">20 APRIL 1992</div> </td> <td style="width: 50%; border: 1px solid black; vertical-align: top;"> Date of Mailing of this International Search Report <div style="text-align: center;">5 MAY 1992</div> </td> </tr> <tr> <td style="border: 1px solid black; vertical-align: top;"> International Searching Authority <div style="text-align: center;">ISA/US</div> </td> <td style="border: 1px solid black; vertical-align: top;"> Signature of Authorized Officer <div style="text-align: center;">  Thurman K. Page </div> </td> </tr> </table>			Date of the Actual Completion of the International Search <div style="text-align: center;">20 APRIL 1992</div>	Date of Mailing of this International Search Report <div style="text-align: center;">5 MAY 1992</div>	International Searching Authority <div style="text-align: center;">ISA/US</div>	Signature of Authorized Officer <div style="text-align: center;">  Thurman K. Page </div>																	
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